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Effects of Azithromycin on Shiga Toxin Production by *Escherichia coli* and Subsequent Host Inflammatory Response

Tatsuki Ohara,[1,2] Seiichi Kojio,[1] Ikue Taneike,[1] Saori Nakagawa,[1] Fumio Gondaira,[1] Yukiko Tamura,[1] Fumitake Gejyo,[2] Hui-Min Zhang,[1] and Tatsuo Yamamoto[1]*

Division of Bacteriology, Department of Infectious Disease Control and International Medicine,[1] and Division of Respiratory Medicine, Department of Homeostatic Regulation and Development,[2] Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

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Shiga toxin (Stx)-producing *Escherichia coli* (STEC) colonizes the human intestinal mucosa, produces Stx from phage, and causes the development of hemolytic-uremic syndrome via Stx-induced inflammatory cytokine production. Azithromycin exhibited strong in vitro activity against STEC without inducing Stx-converting phage, in marked contrast to norfloxacin. Azithromycin decreased the tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and IL-6 production from Stx-treated human peripheral mononuclear cells or monocytes to a greater extent than did clarithromycin. In Stx-injected mice, azithromycin significantly suppressed Stx-induced TNF-α, IL-1β, and IL-6 levels in serum and improved the outcome as assessed by survival rate. In the STEC oral infection experiment using immature mice immediately after weaning (weaned immature-mouse model), all mice died within 7 days postinfection. Azithromycin administration gave the mice 100% protection from killing, while ciprofloxacin administration gave them 67% protection. The data suggest that azithromycin (at least at higher concentrations) has a strong effect on Stx production by STEC and on the Stx-induced inflammatory host response and prevents death in mice. Azithromycin may have a beneficial effect on STEC-associated disease.

Shiga toxin (Stx)-producing *Escherichia coli* (STEC), alternatively called enterohemorrhagic *E. coli*, was identified as an emerging bacterial pathogen of food-borne infections in the United States in 1982 (27). In Japan during 1996, explosive outbreaks of STEC infection occurred, with 17,877 people infected and 12 fatalities (5).

STEC colonizes the intestinal mucosa and elaborates Stx (14, 21). Stx is divided into two subtypes, Stx1 and Stx2 (19), both of which are encoded by prophages lysogenized in STEC strains (17, 23, 24, 35). Stx prophages are induced by agents that damage DNA or inhibit DNA replication. At this stage, accumulated small DNA fragments (such as the Okazaki fragment) bind to and activate the RecA protein (with protease activity), resulting in cleavage of the phage repressor as well as LexA protein (repressor for the *recA* gene) and prophage induction (15, 18, 26). Stx prophage induction is associated with increased Stx gene copy numbers and increased Stx production (18, 20).

The Stx production is followed by the development of abdominal symptoms and by serious systemic disorders such as hemolytic-uremic syndrome (HUS), especially in young children (1, 4, 25).

Stx is a major factor responsible for damaging human endothelial cells and leads to HUS via overproduction of inflammatory cytokines (11, 33, 36). Cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) regulate the expression on the endothelial cell membrane of a glycolipid globotriaosylceramide (Gb3), which serves as the Stx receptor (8, 30, 32, 33).

Treatment regimens that down-regulate such inflammatory cytokines are one option for management of STEC infection. Macrolides including erythromycin, clarithromycin, and azithromycin exhibit immunomodulatory activities, such as inhibition of neutrophil chemotaxis and oxidative burst and the release of proinflammatory cytokines from monocytes (7, 13, 28, 29). However, whether macrolides can modulate Stx-induced inflammatory-cytokine production has not been investigated.

Newer fluoroquinolones such as norfloxacin and ciprofloxacin exhibit strong in vitro activity against STEC. However, they do induce Stx-converting phage (18, 39), resulting in higher levels of Stx production. In this study, we investigated whether azithromycin induces Stx-converting phage and whether azithromycin down-regulates inflammatory cytokine production and prevents death in mice exposed to Stx or challenged with STEC.

MATERIALS AND METHODS

Bacterial strains. The STEC strains used in this study were 208 clinical isolates and included strains derived from outbreaks in Japan in 1996 and from sporadic cases occurring from 1996 to 2001. There were 183 strains of serotype O157:H7, 2 strains of serotype O157:H-, 9 strains of serotype O26:H11, 6 strains of serotype O111:H-, 5 strains of serotype O145:H-, 1 strain each of serotypes O128:H2 and O128:H12, and 1 strain of serotype O86:H- (34). All strains were stored at −80°C in 3% skim milk (Difco Laboratories, Detroit, Mich.) supplemented with 5% glucose (Difco).

Media and bacterial growth. For bacterial growth, nutrient agar (Eiken Chemical, Tokyo), Mueller-Hinton agar (Difco), and Luria-Bertani (LB) agar (Difco) were used as solid media. LB broth (Difco) was used as the liquid medium, which was inoculated and incubated at 37°C for 12 to 18 h with agitation.

* Corresponding author. Mailing address: Division of Bacteriology, Department of Infectious Disease Control and International Medicine, Niigata University Graduate School of Medical and Dental Sciences, 757 Ichibanchou, Asahimachidori, Niigata, Japan. Phone: 81-25-227-2050. Fax: 81-25-227-0762. E-mail: tatsuoy@med.niigata-u.ac.jp.

Antimicrobial agents. The antimicrobial agents (pure substances not including a preservative) were gifts from the manufacturer. They included azithromycin (Pfizer Pharmaceuticals Inc., Tokyo, Japan), clarithromycin (Taisho Pharmaceutical Co., Tokyo, Japan), erythromycin (Shionogi & Co., Osaka, Japan), rokitamycin (Asahi Kasei Corp., Tokyo, Japan), roxithromycin (Aventis Pharma, Tokyo, Japan), oleandomycin (Pfizer Pharmaceuticals Inc., Tokyo, Japan), kitasamysin (Asahi Kasei Corp., Tokyo, Japan), midecamycin (Meiji Seika, Tokyo, Japan), and josamycin (Yamanouchi Pharmaceutical Co., Tokyo, Japan) for macrolides, and norfloxacin (Daiichi Pharmaceutical Co., Tokyo, Japan) and ciprofloxacin (Bayer Yakuhin, Osaka, Japan) for newer fluoroquinolones.

Susceptibility testing. Susceptibility testing of bacterial strains was performed by the agar dilution method with Mueller-Hinton agar by using standard procedures (10).

Preparation of Stx. Stx1 produced from *E. coli* strain 87-27 (carrying the cloned Stx1 gene) and Stx2 produced from *E. coli* strain Tp8 (carrying the cloned Stx2 gene) were purified to homogeneity as described previously (22, 37). Purified Stx1 and Stx2 (0.2 mg of protein per ml) were stored at 4°C in phosphate-buffered saline. The Stx1 and Stx2 preparations contained no detectable endotoxin contamination (less than 0.05 endotoxin unit per ml) as determined by a *Limulus* amebocyte lysate assay.

Stx-converting phage induction. In this experiment, an Stx2-producing STEC strain (serotype O86:H-) isolated from a patient with HUS (strain 1076 [34]) was grown to log phase (ca. 5.0×10^8 CFU/ml) at 37°C in LB broth containing 10 mM $CaCl_2$ and incubated for 30 min in the presence or absence of various concentrations of antimicrobial agents. The bacterial cells were washed and resuspended in the same volume of fresh LB broth and incubated for 2 h. The resultant bacterial culture was divided into two parts.

One part was centrifuged, the supernatant was filtered through a membrane with a pore size of 0.45 μm, and the filtrate was subjected to phage titer determination. For this, 10-fold serial dilutions of the supernatant were made, phages in each dilution were adsorbed onto indicator *E. coli* cells (strain C600), and the cells were incubated overnight at 37°C in LB soft agar containing 10 mM $CaCl_2$. The numbers of phage plaques that developed in the LB soft agar plates (see Fig. 1) were determined. The plaques (at least 20 plaques in each plate) were examined for the Stx2 gene by the PCR assay (21); the data suggested that the plaques were all from the Stx2 phage.

The remaining part of the bacterial culture was subjected to sonification and centrifugation, and the amount of Stx in the supernatant (which was filtered through a membrane with a pore size of 0.45 μm) was determined by the passive latex agglutination test using a VT detection kit (Denka Seiken Co., Tokyo, Japan). The Stx titers (the levels of Stx production) represented the highest dilution (after twofold serial dilutions) to yield positive results.

Preparation of human peripheral blood mononuclear cells and monocytes. Human mononuclear cells were prepared from peripheral blood of healthy volunteers by using Ficoll-Hypaque (Gibco BRL, Grand Island, N.Y.) gradient centrifugation. Adherent monocytes were obtained after incubation in 24-well culture plates (A/S Nunc, Roskilde, Denmark) for 60 min at 37°C in a CO_2 incubator with subsequent washing under high pressure (2).

Cytokine induction by Stx-stimulated human peripheral blood mononuclear cells and monocytes. The human mononuclear cells (1×10^6 to 1.5×10^6/ml) or monocytes (1×10^5/ml), prepared as above, were stimulated with Stx (5 ng/ml) in the presence or absence of antimicrobial agents for 20 h at 37°C in a CO_2 incubator. After incubation, TNF-α, IL-1β, and IL-6 in the supernatants were assayed with TNF-α, IL-1β, and IL-6 kits (Genzyme, Cambridge, Mass.) as specified by the manufacturer.

Stx challenge experiment in mice. Specific-pathogen-free male C57BL/6 mice aged 5 to 6 weeks old and weighing 15 to 20 g were used. They were purchased from Charles River Japan Inc. (Yokohama, Japan) and fed a standard diet and water. The animals were randomized into six groups (10 mice each), five Stx-injected groups and one saline-injected group. The five Stx-injected groups of mice were injected intraperitoneally with 2.8 μg of Stx1 per kg (approximately 55 ng/mouse). This Stx1 dose was chosen because it was shown previously to result in a mortality of >50% between 3 and 4 days after injection. Of the five Stx-injected groups of mice, four were treated with various doses of azithromycin 5 mins after the Stx1 injection. Azithromycin was initially dissolved in ethanol at 12 mg/ml and then diluted 100-fold or more with sterile saline to make a solution of 120 μg/ml (or less). Volumes of approximately 0.5 ml of the azithromycin solutions were administered to the mice to achieve doses of 0.38, 0.75, 1.5, and 3 mg/kg. The Stx-injected, azithromycin-untreated mice received approximately 0.5 ml of sterile saline. Administration of 0.5 ml of sterile saline containing 1% ethenol had no effects on mouse mortality. Survival of the mice was monitored every 24 h after Stx injection. Cumulative mortality was recorded over 7 days after Stx1 injection.

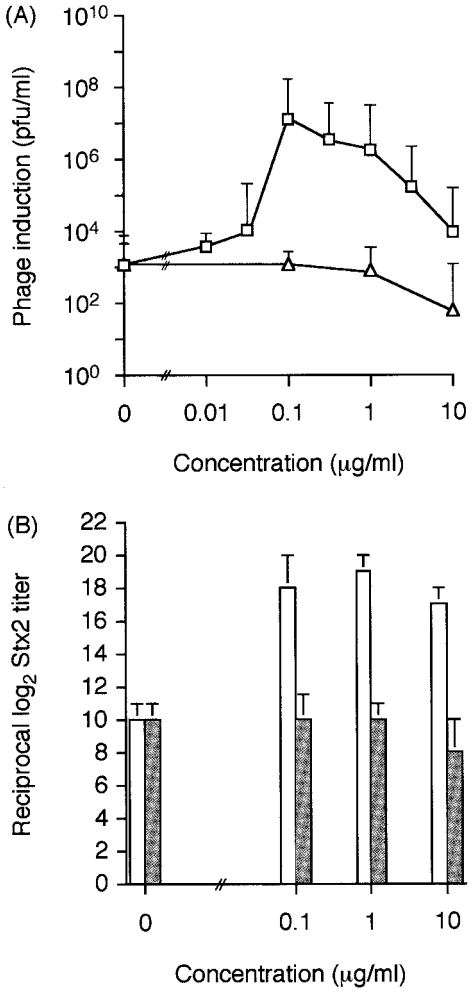

FIG. 1. Stx2 phage induction (A) and Stx2 production (B) in Stx2-producing STEC incubated in the presence or absence of antimicrobial agents. STEC strain 1076 (producing Stx2) was treated with the indicated concentrations of antimicrobial agents for 30 min and then subjected to a 2-h incubation in drug-free medium. (A) Number of Stx2 phage induced. The antimicrobial agents used were norfloxacin (□) and azithromycin (△). (B) Stx2 levels in the bacterial culture after treatment with antimicrobial agents at 0, 0.1, 1, or 10 μg/ml. The antimicrobial agents used were norfloxacin (open bars) and azithromycin (shaded bars).

In parallel experiments (as above), whole-blood samples were obtained from the blood vessels of the eyeground by inhalation at the indicated time points (0, 1.5, 3, 6, and 24 h) after Stx injection. Serum samples were preserved at −30°C until used for measurement of cytokinese levels. Concentrations of TNF-α, IL-1β, and IL-6 in serum were assessed using enzyme-linked immunosorbent assay kits (Genzyme-THCHNE, Minneapolis, Minn.) with a sensitivity of 5.1, 3.0, and 3.1 pg/ml, respectively.

STEC challenge experiment in mice. In mouse infection experiments (designated the weaned immature-mouse model), immature mice (body weight, approximately 9.4 g) immediately after weaning were used. They were specific-pathogen-free male and female C57BL/6 mice and were purchased from CLEA Japan Inc., Tokyo, Japan. The immature mice were divided into four experimental groups (12 animals each): non-STEC infection group (control), STEC infection group, STEC infection and subsequent ciprofloxacin treatment group, and STEC infection and subsequent azithromycin treatment group. STEC (serotype O86 strain 1076) was grown on a nutrient agar plate at 37°C, and the STEC colonies were suspended in PBS at 10^{10} CFU/ml and used for inoculation. In the infection groups, 0.1 ml of the bacterial suspension (10^9 CFU) was orally administered to immature mice via a tube. Ciprofloxacin was dissolved in water and

TABLE 1. MICs of macrolides, norfloxacin, and ciprofloxacin for 208 clinical isolates of Stx-producing *E. coli* strains belonging to serogroups O157, O26, O86, O111, O128, and O145

Antimicrobial agent	MIC (μg/ml)[a]		
	50%	90%	Range
Azithromycin	4	8	2–8
Clarithromycin	32	64	16–128
Erythromycin	64	64	16–128
Rokitamycin	128	≥256	64–≥256
Roxithromycin	128	≥256	64–≥256
Oleandomycin	≥256	≥256	≥256
Kitasamycin	≥256	≥256	128–≥256
Midecamycin	≥256	≥256	≥256
Josamycin	≥256	≥256	≥256
Norfloxacin	0.13	0.13	0.06–0.5
Ciprofloxacin	0.02	0.02	0.01–0.13

[a] 50% and 90%, MICs required to inhibit the growth of 50 and 90% of the strains tested, respectively.

adjusted to 1 mg/ml, and 0.1 ml of the solution was administered; the dose was 10 mg/kg. Azithromycin was prepared with ethanol and adjusted to 100 mg/ml. This ethanol solution was 100-fold diluted with water to 1 mg/ml, and approximately 0.1 ml of this solution was administered; the dose was 10 mg/kg. To antimicrobial agent treatment groups, the agents were orally administered via a tube 24, 48, and 72 h after STEC inoculation. Survival of the immature mice was monitored every 12 h after STEC inoculation.

Statistical analysis. Data concerning cytokine levels were evaluated by Mann-Whitney's U test. Data concerning survival rate in mice were evaluated by Fisher's exact probability test. The level of significance was defined as a P value of <0.05.

RESULTS

In vitro activity against STEC. The MICs of the antimicrobial agents against the STEC strains are summarized in Table 1. Among the macrolides tested, azithromycin showed the greatest activity (MICs, ≤8 μg/ml). The MICs of the newer fluoroquinolones, norfloxacin and ciprofloxacin, were low (≤0.5 μg/ml).

Effect on Stx phage induction. Next, the effect of azithromycin on Stx-converting phage induction was examined. Norfloxacin, whose MIC for strain 1076 was 0.06 μg/ml, markedly induced Stx phage when used at 0.1 μg/ml or more (Fig. 1A). The number of PFU was 10^3- to 10^4-fold greater than for spontaneous phage induction. In accordance with phage induction, marked Stx production was also observed at a norfloxacin concentration of 0.1 μg/ml (Fig. 1B); the Stx titer was increased 512-fold compared with that observed in the absence of antimicrobial agents. In marked contrast, azithromycin did not induce Stx phage (Fig. 1A) and did not stimulate Stx production (Fig. 1B). Similar results were obtained with Stx1 phage induction and Stx1 production in experiments using an Stx1-producing STEC strain (data not shown).

Inhibitory effect on cytokine induction by Stx-stimulated human peripheral blood mononuclear cells and monocytes. Stx stimulates the production of TNF-α and IL-1β from human peripheral blood monocytes. The effects of azithromycin on modulating such Stx-induced inflammatory cytokine production were then examined.

Data for Stx1 stimulation of human mononuclear cells are shown in Fig. 2. Azithromycin inhibited the Stx1-stimulated cytokine production in a dose-dependent manner. The percent

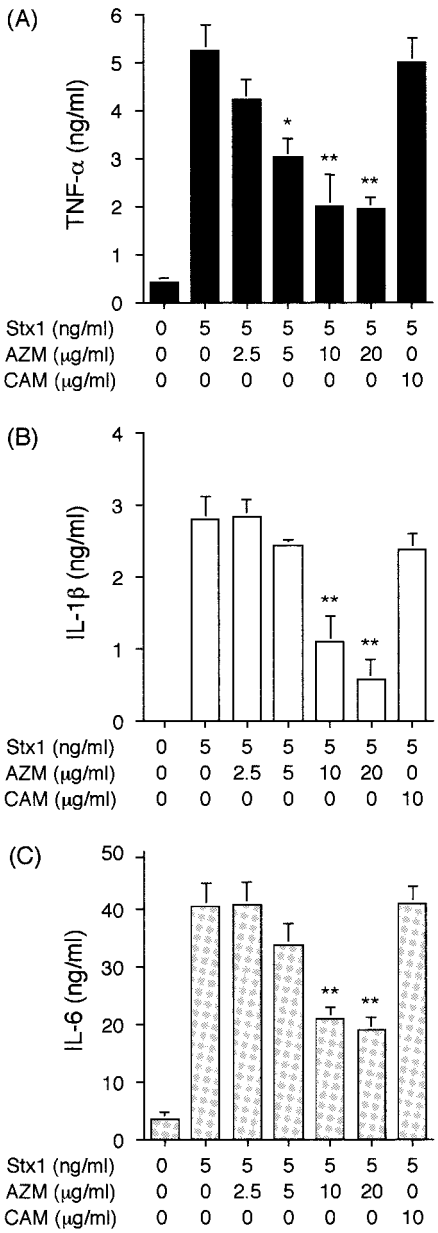

FIG. 2. Inhibition of cytokine production from Stx1-stimulated human peripheral blood mononuclear cells by azithromycin and clarithromycin. Human mononuclear cells were stimulated with Stx in the presence or absence of azithromycin (AZM) or clarithromycin (CAM). *, $P < 0.05$; **, $P < 0.01$, compared with Stx-stimulated mononuclear cells in the absence of antimicrobial agents. Similar inhibitory effects were observed even when human monocytes were used, although the cytokine levels were slightly lower compared with the data shown in this figure.

inhibition at an azithromycin concentration of 10 μg/ml was 61.7% for TNF-α, 60.4% for IL-1β, and 47.7% for IL-6. With clarithromycin, much less or no significant inhibition was observed at 10 μg/ml ($P < 0.01$); the inhibition was 4.7% for TNF-α, 14.8% for IL-1β, and 1.3% for IL-6.

Similar inhibitory effects of azithromycin were obtained even when human monocytes were used or when Stx2 stimulation was used instead of Stx1 stimulation; inhibition of TNF-α in-

duction by azithromycin (10 μg/ml) in human monocytes was 64.5% for Stx1 and 66.6% for Stx2, and inhibition of Stx2 stimulation of IL-1β and IL-6 was 91.5 and 80.6%, respectively.

Inhibitory effect on serum cytokine levels. The effect of azithromycin on serum cytokine levels after Stx1 stimulation was examined in mice. First, we determined the induction of cytokines in mouse sera by Stx1. The TNF-α level generally peaked around 1.5 h and IL-1β and IL-6 usually appeared around 3 h after Stx1 injection (data not shown). Therefore, we evaluated the effects of azithromycin on Stx1-induced TNF-α, IL-1β, and IL-6 production at 1.5, 3, and 3 h after injection, respectively.

The effects of azithromycin on serum cytokine levels after Stx1 stimulation in mice are shown in Fig. 3. Treatment with various doses of azithromycin resulted in a marked decrease in Stx1-induced TNF-α production (Fig. 3A). Compared with the TNF-α levels in saline-treated mice, azithromycin at doses as low as 0.38 mg/kg significantly inhibited Stx1-induced TNF-α production ($P < 0.05$).

The degree of enhancement of IL-1β production in mice injected with Stx1 was low (Fig. 3B). We determined the concentration of IL-1β in serum 3 h after treatment with various doses of azithromycin. Azithromycin at 0.38 mg/kg significantly reduced the peak concentration of IL-1β in serum compared with that in saline-treated mice ($P < 0.05$).

Stx1 showed a greater enhancement of IL-6 than of TNF-α and IL-1β production in mouse sera (Fig. 3C). Increased concentrations of IL-6 were observed 1.5 h after injection of Stx1 and reached a peak after 3 h. We found that azithromycin at 3 mg/kg also significantly inhibited Stx1-induced IL-6 production ($P < 0.05$). However, at doses of less than 3 mg/kg, this agent had no significant inhibitory effect (Fig. 3C). In these experiments, the administration of azithromycin alone did not produce increases in the concentration of TNF-α, IL-1β, or IL-6 (data not shown).

Protective effect against Stx challenge. The protective effect of azithromycin against Stx1 challenge was examined in mice. We compared the lethality of Stx1 by using C57BL/6 mice with or without azithromycin treatment (Fig. 4). Stx1 injection (2.8 μg/kg) resulted in 100% mortality in saline-treated mice (0% survival). In contrast, treatment with azithromycin at 3 mg/kg resulted in a significantly higher rate of survival (40%) than that in the Stx-injected, saline-treated controls ($P < 0.05$). However, lower doses of azithromycin (0.38, 0.75, and 1.5 mg/kg) resulted in a survival of 20%, which was not significantly different from that of the Stx-injected, saline-treated controls ($P > 0.05$).

Protective effect against STEC challenge. The STEC oral-infection experiment was performed using immature mice immediately after weaning. Survival of the immature mice was monitored every 12 h after STEC inoculation (Fig. 5). In the STEC infection group, the immature mice began to die 48 h after inoculation and all mice died within 5 days after inoculation. In contrast, in the ciprofloxacin treatment group, death was observed 60 h after inoculation but the mortality rate was only 33% (survival rate, 67%) 1 week after inoculation; this was the case even 2 weeks after inoculation. In the azithromycin treatment group, no deaths were observed and all animals were alive even 2 weeks after inoculation.

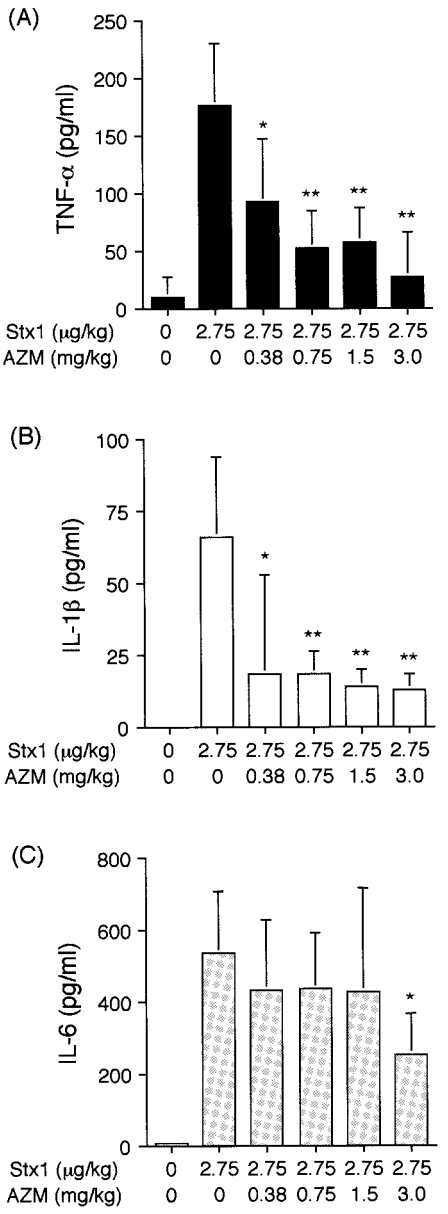

FIG. 3. Dose-response effects of azithromycin on TNF-α (A), IL-1β (B), and IL-6 (C) levels in serum after Stx1 stimulation in mice. Groups of 10 mice were injected intraperitoneally with 55 ng of Stx1. Treatment (intraperitoneal injection) with various doses of azithromycin (AZM) or saline was carried out 5 min after the Stx1 injection. (A) TNF-α production 1.5 h after Stx1 stimulation in mice; (B) IL-1β production 3 h after Stx1 stimulation in mice; (C) IL-6 production 3 h after Stx1 stimulation in mice. Data are means ± standard deviations (error bars). *, $P < 0.05$; **, $P < 0.01$, compared with those of Stx-injected, azithromycin-untreated mice.

DISCUSSION

Inflammatory cytokines up-regulate the expression of the receptor for Stx on endothelial cells and allow the endothelial cells to be more sensitive to the toxic effect of Stx (32, 33). Elevated concentrations of TNF-α and IL-6 in plasma have been reported in HUS patients, and the degree of HUS is closely related to the levels of inflammatory cytokines (12, 16).

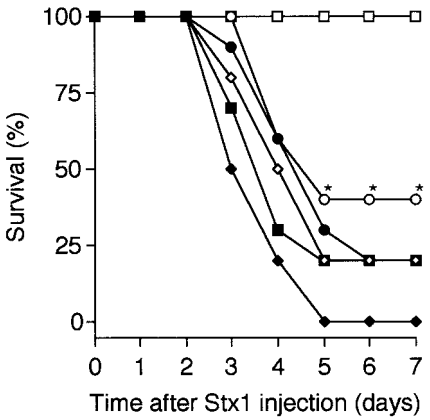

FIG. 4. Lethality of Stx1 in mice with or without azithromycin treatment. Groups of 10 mice were injected intraperitoneally with 55 ng of Stx1. Treatment (intraperitoneal injection) with various doses of azithromycin (or saline) was carried out 5 min after the Stx1 injection. Mice injected only with saline (with no Stx injection) were used as controls (□). Doses of azithromycin were 0 mg/kg (◆), 0.38 mg/kg (■), 0.75 mg/kg (◇), 1.5 mg/kg (●) and 3 mg/kg (○). *, $P < 0.05$, compared with the Stx-injected, untreated controls (◆).

Therefore, inflammatory cytokines are thought to be important in modifying the disease caused by STEC infection.

The roles of antimicrobial agents in the prevention and amelioration of HUS remain controversial, and an optimal treatment regimen for STEC infection has not been established (9, 31). In this study, we demonstrated that azithromycin has a strong effect on Stx production by STEC. Azithromycin inhibited the in vitro growth of STEC strains and did not induce Stx-converting phage or stimulate the production of Stx at a wide range of concentrations in vitro. This was in sharp contrast to newer fluoroquinolones such as norfloxacin and ciprofloxacin (18, 39), which markedly induced Stx-converting phage and stimulated the production of Stx.

In addition to the merits of azithromycin reported above, the results of the present study suggested that azithromycin is capable of inhibiting the Stx1- or Stx2-induced production of inflammatory cytokines (TNF-α, IL-1β, and IL-6), which are considered to be essential for the development of HUS. Preliminary data suggested that azithromycin also inhibited Stx1- or Stx2-induced production of IL-8 from human monocytes by 67 to 78%. Azithromycin treatment at 3 mg/kg resulted in the greatest reduction in cytokine levels and improved outcome as assessed by monitoring the survival rate. Although further studies are necessary to investigate the mechanism of this effect, we speculated that improvement of the survival of mice may in part be due to the anti-inflammatory effect of azithromycin.

In healthy volunteers, azithromycin achieved maximum concentrations in serum of 0.09 μg/ml following a single oral dose of 125 mg (approximately 2.2 mg/kg), 0.24 μg/ml following a single oral dose of 250 mg (approximately 3.6 mg/kg), 0.58 μg/ml following a single oral dose of 500 mg (approximately 8.3 mg/kg), and 0.74 μg/ml following a single oral dose of 1,000 mg (approximately 15.4 mg/kg) (3). However, concentrations of azithromycin in tissue are much higher than are concentrations in serum. For instance, after two 250-mg (approximately 3.5-mg/kg) oral doses given 12 h apart, peak azithromycin concentrations exceeded 3 mg/kg in the prostate, tonsils, and many other tissues (6). Therefore, the dose of 3 mg/kg used in this study for mice seems to be relevant to human dosing levels.

We previously reported that a Chinese medicine, anisodamine (a vasoactive alkaloid drug extracted from a Chinese herb), inhibits TNF-α, IL-1β, and IL-8 production by Stx1-stimulated human peripheral blood monocytes and increases the survival of Stx1-treated mice (38). The protective effect of azithromycin, observed in this study, was at a level similar to or slightly lower than that of anisodamine. Anisodamine, however, had no effects on the in vitro growth of STEC strains (MICs, ≥256 μg/ml).

In this study, we also performed the infection experiment using immature mice immediately after weaning (designated the weaned immature-mouse model). Immature mice immediately after weaning were susceptible to STEC. The animals began to die 2 days after oral inoculation, and all animals died within 5 days after inoculation. Investigation of the pathology of STEC infection and the cause of death in weaned immature mice is in progress. When ciprofloxacin was administered in this experimental system, the mortality after 1 week (or even after 2 weeks) was 33% and the survival rate was 67%. The infection-preventing effect of azithromycin was higher than that of ciprofloxacin ($P < 0.05$), and the survival rate was 100% even 2 weeks after inoculation. Ciprofloxacin administration induced Stx phages, which may have been the cause of the lower infection-preventing effect of ciprofloxacin than that of azithromycin.

In conclusion, the present study demonstrated that azithromycin (at least at higher concentrations) has a strong effect on Stx production by STEC and the Stx-induced inflammatory host response and prevents death in mice exposed to Stx or

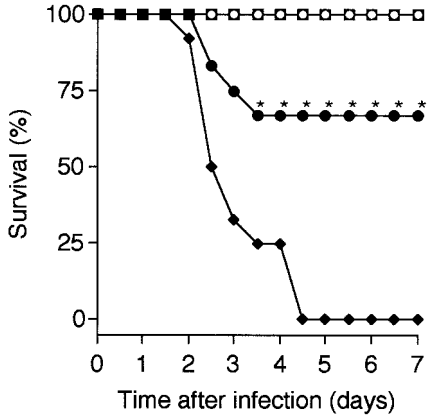

FIG. 5. Effect of antimicrobial agents on lethality after oral infection of mice with STEC. Three groups of immature mice (12 mice each) were infected orally with 10^9 CFU of STEC strain 1076 (producing Stx2) via a tube on day 0 in this figure. In an additional group of 12 immature mice, the same volume of phosphate-buffered saline was given orally instead of STEC (□). At 1, 2, and 3 days after STEC inoculation, the two infected groups of mice were treated with azithromycin (10 mg/kg/day) (○) or ciprofloxacin (10 mg/kg/day) (●). The remaining infected group of mice (◆) was not treated with antimicrobial agents. Survival of mice was monitored every 12 h after STEC inoculation. *, $P < 0.05$, compared with azithromycin-treated, STEC-infected mice.

STEC. Azithromycin may have a beneficial effect on STEC-associated disease. Further studies to investigate the potential usefulness of azithromycin are necessary.

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